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# INDUCED MYOGENIC ACTIVITY IN THE NEUROGENIC HEART OF *LIMULUS POLYPHEMUS*

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Many recent studies have suggested that the classification of neurogenic and myogenic hearts is more ambiguous than was once believed. In general, the hearts of chordates and molluscs were thought to be myogenic while the hearts of arthropods and annelids were thought to be neurogenic (Prosser and Brown, 1961). A notable exception was the heart of the cladoceran, *Daphnia*, which was thought to be myogenic (Baylor, 1942). More recently, myogenic activity has been reported in the heart of other arthropods, notably a moth (*Hyalophora cecropia*, McCann, 1963), an isopod crustacean (*Megaligia crotica*, Ai, 1966) and a cockroach (*Periplaneta americana*, Miller, 1969).

The heart of the mature horseshoe crab, *Limulus polyphemus*, was shown to be neurogenic by the elegant experiments of Carlson (1904a). He demonstrated that if the ganglion was cut, leaving the muscle intact, the heart established different rates of contraction on either side of the cut. If the muscle was cut, even in several places, leaving the ganglion intact, all portions of the muscle beat in synchrony. Upon removal of the ganglion, the heartbeat ceased. However, Carlson (1904b, 1907) later reported that *Limulus* heart apparently had a latent myogenic mechanism that was seen upon immersion of a deganglionated heart in what he described as "isotonic" sodium chloride solution (600 mM). This activity was never seen in normal physiological solution and could be abolished by addition of a small amount of calcium chloride to the isotonic sodium chloride (Carlson, 1904b).

Following Carlson's work, a number of investigators contested his findings of absence of myogenic activity when a deganglionated *Limulus* heart was immersed in physiological solution or sea water (reviewed by Krijgsman, 1952). However, it was Heinbecker (1933, 1936) that first demonstrated convincingly that myogenic activity could be induced in *Limulus* heart under nearly physiological conditions. He reported that a deganglionated heart immersed in sea water and inflated with air or sea water would again begin to beat.

The present report is concerned with a preliminary investigation of the electrophysiological basis for both the sodium chloride-induced and the stretch-induced myogenic activity.

## Methods

Experiments were conducted during summer, 1969 at the Marine Biological Laboratory, Woods Hole, Massachusetts. Large males and females, measuring 15–25 cm across the carapace, were kept in running sea water until used. Hearts were isolated as previously described (Abbott, Lang and Parnas 1969a), pinned out in a wax-filled lucite chamber, and their cardiac ganglia carefully removed. Subsequent preparation differed for the two types of myogenicity. For experiments

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on sodium chloride myogenicity, hearts were pinned out either with their myocardium intact or after making a ventral midline incision. There was no apparent difference in activity by these two methods and the latter method was preferred since the heart could be mounted lumen side up, permitting easier penetration of fibers by microelectrodes. The hearts were then immersed in 600 mM sodium chloride (Carlson 1904b) or in the concentration of sodium chloride found in *Limulus* physiological solution (444 mM, Chao, 1933).

A different method of preparation was used for inflation-induced myogenicity. A cannula was inserted into the anterior end of a deganglionated heart and securely fastened with silk thread. The heart was immersed in sea water or *Limulus* physiological solution (444 mM NaCl, 37 mM $CaCl_2$, 9 mM KCl, Chao, 1933) and perfused with air from a pump. When spontaneous contractions began, both ends of the heart were tied off and pinned to the bottom of the chamber, keeping the preparation immersed. Usually the heart remained inflated throughout the entire experiment, the ostia being closed by the internal pressure. If air leaked out, the heart always ceased contracting regardless of how long it had been beating myogenically.

Intracellular electrical activity was recorded by KCl-filled microelectrodes (10–15 MΩ) suspended by fine tungsten wire (0.001″) with a small drop of paraffin.

## Results

The normal contraction of *Limulus* myocardial muscle results from a tetanic volley of junctional potentials (j.p.'s) which summate and cause a sustained depolarization of the muscle for about 1.0–1.5 sec (Lang, Abbott and Parnas 1967). These potentials (Fig. 1) reach a maximum of 30–35 mv and are never overshooting (resting potentials, 35–45 mv).

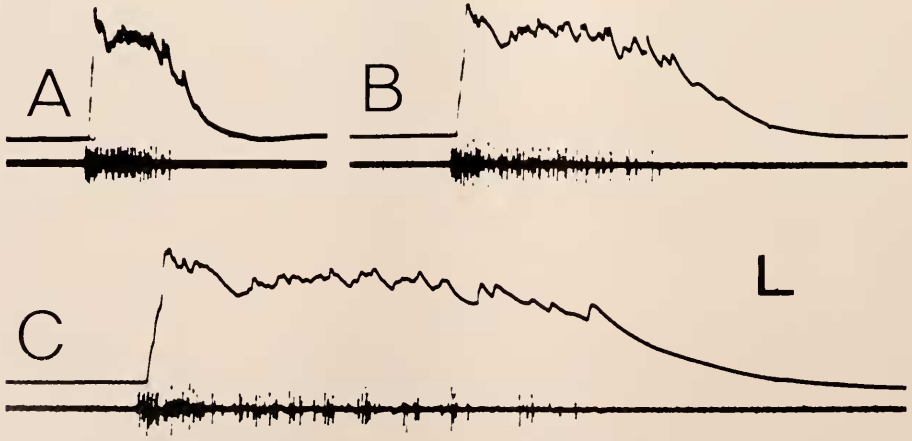


Figure 1. Electrical activity of an intact, spontaneously beating *Limulus* heart. Intracellular muscle (upper trace) and extracellular ganglionic (lower trace) recordings show that intracellular activity is due to summation and sustained depolarization from a volley of j.p.'s; calibration: vertical, A–C upper trace, 10 mv; lower trace, 40 μv; horizontal, A, 500 msec, B, 200 msec, C, 100 msec.

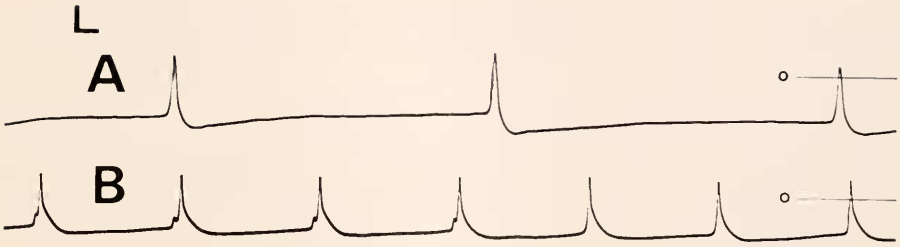


FIGURE 2. Low calcium induced myogenic activity in deganglionated *Limulus* hearts; intracellular activity from two different hearts (A and B) immersed in 0.6 M NaCl. Note pacemaker potentials. Zero potential indicated; calibration: 20 mv, 500 msec.

*Sodium chloride myogenicity*

When a *Limulus* heart was deganglionated, pinned out, and immersed in 600 mM sodium chloride, rhythmic local contractions began rather suddenly within 10–20 min. This activity lasted longer than 30 minutes but was seldom sufficiently coordinated to result in a synchronous contraction of most or all of the heart.

Impalement of muscle cells in spontaneously contracting segments revealed slowly depolarizing pacemaker potentials which induced rhythmic spiking activity (Fig. 2). Resting membrane potentials were 35–45 mv. Spikes were usually overshooting, and had long durations, never lasting less than 100 msec and often lasting several hundred msec. Often spikes of long duration and small amplitude were observed. Their slow rise time and diminished height suggested that they may have been decrementally conducted from a distant spiking site (see Rulon, Hermsmeyer and Sperelakis, 1971).

The parameters of spike duration and spike height were variable from one recording site to another in a given heart as well as at a given recording site over short periods of time. In addition, two spikes were often recorded sequentially from a single fiber, each having unique parameters of duration and height (Fig. 2B).

Spike height was dependent on external sodium ion concentration. In 444 mM sodium chloride, the same concentration as found in *Limulus* physiological solution, spikes were never overshooting (Lang, 1970; Rulon, Hermsmeyer and Sperelakis, 1970). Decreasing external sodium to 225–250 mM, and replacing with the osmotic equivalent of sucrose, resulted in a decrease in spike height to the point where spikes either became very small or disappeared. Thus spike height was shown to be proportional to external sodium concentration in the range from normal (444 mM) down to 45% of normal, where activity ceased (Rulon, *et al.*, 1970). Further increase in the external sodium concentration resulted in further increase in spike height. In 600 mM sodium chloride, spikes overshoot zero potential by 10–20 mv (Fig. 2).

Addition of double the physiological concentration of potassium (18 mM) to the 600 sodium chloride had no appreciable effect on the electrical or mechanical activity. However, addition of 2–3 mM calcium chloride to the 600 mM sodium chloride eliminated the myogenic activity almost immediately.

Tetrodotoxin (TTX), a potent inhibitor of sodium dependent spikes in excitable tissues, failed to block the myogenic activity when added to the 600 mM

sodium chloride even when relatively high concentrations ($10^{-5}$ M) were employed. Likewise, $10^{-4}$ M procaine, a local anesthetic, had no effect on the myogenic activity.

A characteristic of the sodium chloride myogenicity was the presence of a latent period between immersion of a deganglionated heart in the solution and the time it began to contract. Hearts that were deganglionated and kept in *Limulus* saline for 30 minutes still exhibited the latent period before beginning to contract after immersion in 600 mM sodium chloride. The latent period was also present if an intact, neurogenically beating heart was immersed in 600 mM sodium chloride. The initial effect in this case was an increase in the heart rate and a decrease in the coordination of the beating (Fig. 3B). After five minutes, there was evidence of a regenerative response of the muscle membrane in the form of overshooting spikes on top of the summated j.p.'s (Fig. 3C). After 7 minutes, every other burst from the ganglion had an overshooting spike on top of the neurogenically induced activity (Fig. 3D). These results suggested that a period of synaptic inactivity was not necessary for the myogenic activity but that a certain period of immersion in the calcium chloride solution was necessary, probably to wash out available calcium from the muscle. Whatever the precise cause of the change

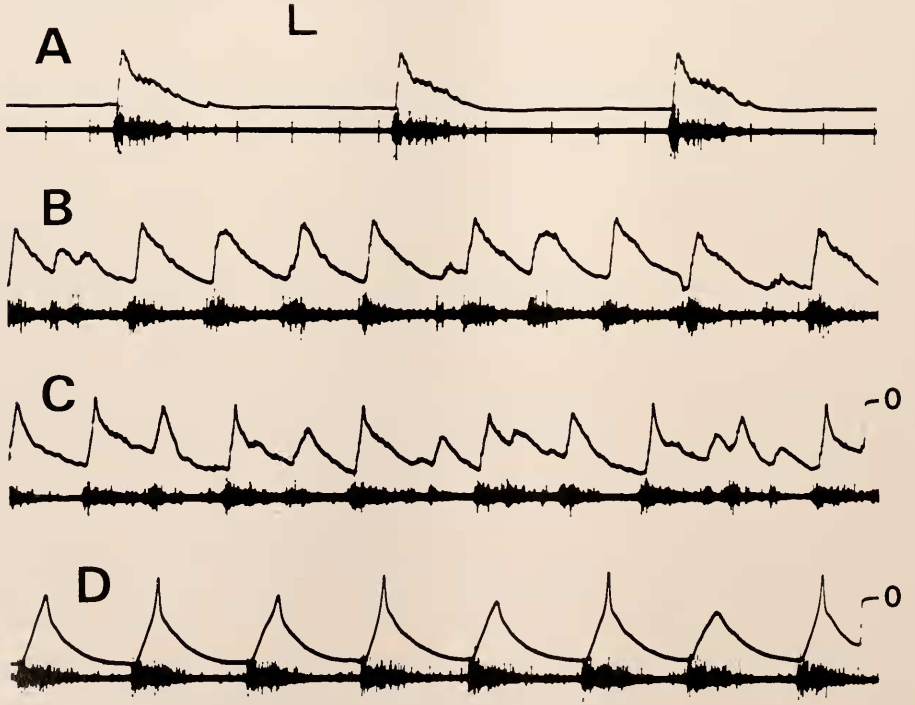


FIGURE 3. Effect of 0.6 M NaCl on spontaneously beating heart. Intracellular (upper trace) and extracellular ganglionic (lower trace) activity; A, control; B, two minutes after immersion in 0.6 M NaCl; C, after second wash in 0.6 M NaCl at 5 min; there appear to be spikes on the peaks of some of the intracellular bursts; D, after third wash at 7 min; every second burst has an overshooting spike; calibration: 10 mv, 500 msec.

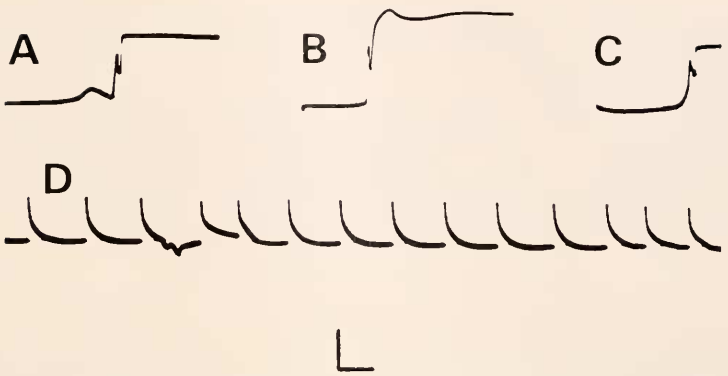


FIGURE 4. Stretch-induced myogenic activity in a deganglionated *Limulus* heart. Intracellular recording; A–C, the electrode was dislodged due to the mechanical activity, immediately after the peak of the spike; D, train of spikes in a muscle fiber that was not contracting; calibration: 20 mv, 500 msec.

in membrane characteristics, its time course seemed to be reflected in the records of Figure 3C–D. In Figure 3C, spikes are triggered only on the peak of the largest of depolarizations, indicating that they had a high threshold. In addition the fact that not all of the large depolarizations in Figure 3C–D triggered a spike may be evidence of a long refractory period at this stage in development of the myogenic activity.

*Stretch-induced myogenicity*

When a deganglionated heart was inflated with air and immersed in *Limulus* physiological solution or sea water, it began to contract within 20 min. The activity consisted of a simultaneous contraction of the entire heart circumference, in part of a segment, which occluded the lumen. The contraction wave passed peristaltically in both directions from the point of origin. Normally the contraction began in segment 2, between the first and second pairs of ostia, and was conducted at the rate of 2–4 cm/sec. Activity was observed for over two hours, as long as the heart remained inflated. If the inflation was released, the contractions stopped.

Impalement of the myocardial cells during stretch-induced myogenicity proved to be difficult. In order to induce the contractions, it was necessary to inflate the heart to 2–3 times its normal diastolic diameter. During contraction, the lumen was nearly occluded causing downward movement of the heart wall of about 1.5–2 cm. Consequently, the microelectrode was almost invariably dislodged as the muscle contracted, immediately after a spike (Fig. 4A–C). In a few experiments, impaled fibers exhibited spikes at a rate of 2–3/sec, unaccompanied by contractions (Fig. 4D), thus the microelectrode was not dislodged during this activity.

Membrane resting potentials of inflated hearts did not differ appreciably from those found in a spontaneously beating neurogenic preparation. Stretch-induced spikes were 30–40 mv height and were never overshooting. Spike rise times were 5–10 msec, although spikes with slower rise times were frequently observed.

Pacemaker potentials were absent or very small. Neither TTX ($10^{-5}$ M) nor procaine ($10^{-4}$ M) had any effect on the electrical or mechanical activity of the stretch-induced myogenicity.

Attempts were made to induce myogenicity by stretching a deganglionated heart on a large glass rod or over an inflated balloon in order to eliminate the vigorous concentration. These procedures were unsuccessful even if the hearts were first induced to beat myogenically by inflating with air. It appeared that ability to shorten was necessary for stretch-induced myogenic activity.

## Discussion

Myogenic activity has been shown to occur in several arthropod hearts (Baylor, 1942; McCann, 1963; Ai, 1966; Miller, 1969). The presence of a latent myogenic pacemaker mechanism in *Limulus* heart is not surprising when one considers the ontogeny of the species. Carlson and Meek (1908) and Prosser (1942) have shown that the embryonic *Limulus* heart is myogenic from day 21, when the beat is first visible, until day 30 when ganglion cells are first histologically demonstrable. During this period the heart beats peristaltically, the contraction beginning in the anterior end (Lang, unpublished observation) in contrast to the adult heart where the beat begins in one of the posterior segments (Carlson, 1904a).

### *Sodium chloride myogenicity*

Carlson (1904b) observed that a normally quiescent, deganglionated *Limulus* heart would again begin to contract if immersed in isotonic sodium chloride solution. These contractions were shown to be caused by sodium-dependent spikes initiated in the myocardium (Lang, 1970; Rulon *et al.*, 1970). Addition of just a few mM calcium chloride inhibited the contractions (Carlson, 1904b) and the electrical activity (Lang, 1970). The contractions in this myogenic state were seldom coordinated. Segments usually beat independently of adjacent segments. Occasionally two or three adjacent segments beat peristaltically or in near synchrony. It was uncertain whether the activity was conducted electrically or mechanically but it may have been a combination of both. The muscle was sensitive to mechanical stimulation; lightly touching the myocardium caused local contractions. However, decrementally conducted potentials were often observed, so conduction of a regenerative potential throughout the heart muscle was not always complete (*cf.* Rulon *et al.*, 1971). On the other hand, spike potentials probably travel for some distance decrementally, perhaps even from fiber to fiber through the intercalated discs known to occur between them (Jordan, 1917; Lang, 1970; Sperelakis, 1971). If fiber to fiber conduction was present, it probably occurred between longitudinally adjacent fibers (*i.e.* in a circular direction in the heart) since intercalated discs have not been shown to occur between laterally adjacent fibers (*i.e.* in the longitudinal direction of the heart) in *Limulus*. In addition, Parnas and co-workers failed to find electronic coupling between laterally adjacent fibers (Parnas, Abbott and Lang, 1969).

The precise mechanisms involved in the sodium chloride myogenicity are not known. Certainly, the main prerequisite is the absence of calcium ions in the bathing solution. Apparently, concentrations of calcium below 2–3 mM effect the

membrane sufficiently to cause periodic transient increases in sodium conductance. This interpretation is consistent with the results of others who also found that calcium effects stability of the membrane of excitable tissue. Low calcium levels were shown to cause repetitive firing in frog (Brink, Bronk and Larrabee, 1946) lobster (Adelman and Adams, 1959) and spider nerves (Rathmayer, 1965). In addition, calcium was shown to affect permeability to sodium ions in squid giant axon (Frankenhaeuser and Hodgkin, 1957; Guttman and Barnhill, 1970) and to have a direct effect on membrane resistance in lobster muscle fibers (Werman and Grundfest, 1961). More specifically, calcium and sodium were shown to compete for the same sites in the membrane of rat (Gage and Quastel, 1966) and frog (Birks, Burstyn and Firth, 1968) neuromuscular junctions and in frog heart (Lüttgau and Niedergerke, 1958). Further work on the present preparation might shed light on whether the ratio of sodium: calcium is important for activity, whether other monovalent cations can substitute for the sodium, and the importance of permeable monovalent anions in sustaining the activity.

*Stretch-induced myogenicity*

Myogenic activity of adult *Limulus* heart in physiological solution was first convincingly demonstrated by Heinbecker (1933). This activity resulted after internal pressure was increased. The resulting contractions traveled peristaltically, starting in one of the anterior segments, as in the beat of the embryonic Limulus heart. Spread of the contraction was slow (2–4 cm/sec) and the muscle exhibited spiking activity. Again, the mode of conduction in the preparation was uncertain. Spike potentials were nearly uniform in size for all preparations, suggesting active conduction of the spike or at least a uniform conductance change throughout the muscle. However spread of activity via mechanical stimulation of the membrane cannot be ruled out since the ability to contract appeared to be a prerequisite for stretch-induced myogenicity.

Initiation and maintenance of this type of activity appears to be dependent on stretch of the muscle. Stretch is known to affect the *Limulus* heart muscle, causing increased contraction for a given stimulus in a deganglionated heart (Abbott, Lang, Parnas, Parmley and Sonnenblick 1969b). It was uncertain whether this effect of stretch was on the muscle membrane or on the contractile apparatus directly but in light of the present results, it seems likely that stretch can have an effect directly on the myocardial membrane.

It might be suggested that both the sodium chloride myogenicity and the stretch-induced myogenicity had their primary effect by exciting the motor nerves of the heart, which in turn excited the muscle. This appears unlikely since TTX, which was shown to block the motor axons in *Limulus* heart (Abbott *et al.* 1969b) did not affect either type of myogenic activity. But this in itself is somewhat surprising since the spikes, at least in the sodium chloride-induced myogenicity, were shown to be dependent on external sodium concentration. However, another recent report has shown that TTX is not always effective in blocking sodium dependent spikes (Redfern, Lundh and Thesleff 1970). In this regard, it would be of interest to know whether TTX can block the low calcium-induced spiking in other excitable tissues and whether it can block the myogenic beat of the embryonic *Limulus* heart.

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## Summary

Myogenic activity could be initiated in a deganglionated *Limulus* heart in two different ways. If immersed in "isotonic" sodium chloride (600 mM) the heart began to contract locally in 5–10 minutes. These contractions were seldom coordinated and were due to overshooting spikes of long duration (100 msec.). Spike height was a function of the external sodium ion concentration. The activity was completely abolished upon addition of 2–3 mM $CaCl_2$.

A second type of myogenic activity could be initiated by inflating a deganglionated heart with air and immersing it in sea water or physiological solution. The contractions began in 10–20 min in one of the anterior segments and travelled peristaltically in both directions. Activity was caused by non-overshooting spikes.

Both types of myogenic activity were resistant to tetrodotoxin (TTX, $10^{-5}$ M) and procaine ($10^{-4}$ M).

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